

# Distinct Circulating Recombinant HIV-1 Strains Among Injecting Drug Users and Sex Workers in Afghanistan

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## Abstract

Little information is available regarding a circulating HIV genotype among high-risk groups in Afghanistan; we describe HIV genotypes among injecting drug users (IDUs) and sex workers (SWs) in four Afghan cities. Participants completed behavioral questionnaires and HIV testing. Western blot-confirmed specimens had peripheral mononuclear blood cells isolated for genotyping. Analysis of recombinants was done by bootscanning and manual sequence alignment. The single SW sample harbored a CRF01\_AE strain. Of 10 IDUs available for analysis, all were CRF35\_AD and from Hirat. Analyzed subregions (*gag* p17 and *env* C1-C5) revealed close homology between the Hirat specimens. Three distinct subclusters comprising two or three strains were identified, whereas two other strains were generally equidistant from previously identified Kabul strains. Results suggest that the nascent HIV epidemic among IDUs in Hirat is largely, if not entirely, subtype CRF35\_AD, and the close homology suggests recent infection; harm reduction should be supported to avert further transmission.

## Introduction

HIV EPIDEMICS DRIVEN by injecting drug use are emerging in multiple cities in Iran and Pakistan.<sup>1,2</sup> Assessment of HIV-1 genotype among injecting drug users (IDUs) performed in both countries has identified a predominance of subtype A strains, with subtype B strains identified in Iran among patients infected through blood transfusion.<sup>3-5</sup> Behavioral assessments of these high-risk populations in multiple settings have identified a strong potential for bridging of the HIV epidemic from IDUs to other groups, chiefly sex workers (SWs).<sup>1,6-8</sup>

HIV genotype information may be useful to describe transmission linkages between risk groups. In analyzing HIV-1 strains from IDUs in Iran, Tagliamonte *et al.*<sup>5</sup> identified greater homology within the isolated subtypes from Tehran than those from Mashad, providing some objective insight into the duration of the respective epidemics in the two cities and interactions between their IDU networks.

Afghanistan shares borders with Pakistan and Iran and has had a substantial influx of repatriating refugees from those countries in the last 7 years. Afghanistan is also the leading

global producer of opium and has experienced burgeoning problem drug use, including transitions from noninjecting to injecting of opiates and pharmaceuticals.<sup>9</sup> The interplay between IDUs and other vulnerable populations may lead to spread of HIV in ways distinct from those of neighboring countries. A nascent HIV epidemic among IDUs in Kabul has been reported, with genetic analysis of four samples identifying the unique recombinant CRF35\_AD.<sup>10</sup> However, few data are available regarding other risk groups or IDUs in other areas of Afghanistan, a country with diverse populations. As a part of this study, HIV seroprevalence and high-risk behaviors of IDUs and SWs were assessed in three additional Afghan cities, with genetic analysis of confirmed HIV specimens. Herein, we present and characterize sequenced HIV-1 genotypes from high risk groups in the cities of Hirat, Jalalabad, Kabul, and Mazar-i-Sharif, Afghanistan.

This cross-sectional study conducted between September 2006 and January 2008 surveyed SWs from Kabul, Jalalabad, and Mazar-i-Sharif, and IDUs in Jalalabad, Mazar-i-Sharif, and Hirat. All cities have functioning Ministry of Public Health Voluntary Counseling and Testing Centers (VCTs) and groups working with either or both of the high-risk

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populations of interest. Before data collection, this study was approved by the institutional review boards of the University of California, San Diego; the Walter Reed Army Institute of Research; the U.S. Naval Medical Research Unit 3 in Cairo, Egypt; and the Ministry of Public Health of the Islamic Republic of Afghanistan.

Eligible participants were individuals aged 18 years or older reporting injecting drugs (confirmed through injection stigmata) or sex work within the past 6 months and able to provide informed consent. IDUs and self-identified SWs using existing programs were recruited through outreach workers known to them. Those interested in participation were brought to the program office or, if not possible, to the study office and met in a confidential room by a gender-matched study representative. No data were recorded on those declining or ineligible for study entry. At each enrollment site, the study representatives obtained written informed consent, administered the questionnaire, provided pre-test counseling, performed whole-blood ra-

pid testing, and completed posttest counseling. The questionnaires assessed sociodemographics, travel history, sex-work history, and activity for SWs and sexual-activity history for IDUs, past and current drug-use behaviors, past and current STI symptoms, condom (men and women) and contraceptive (women only) use, and knowledge about HIV. SW participants received a nonmonetary gift of hygiene and grooming supplies (*e.g.*, shampoo, toothpaste), whereas IDU participants received a small nonmonetary gift of hygiene items (*e.g.* razor, soap) with referrals for addiction treatment and Needle and Syringe Programs (NSPs) on request.

Rapid HIV-1 antibody testing was performed with Abbott Determine® (Abbott Diagnostics Japan, Tokyo, Japan); participants with a positive Determine® HIV test underwent sequential testing with a second rapid test, HIV (1 + 2) Antibody Colloidal Gold (KHB Kehua Shanghai). Repeatedly positive rapid HIV tests were confirmed with Western Blot (HIV BLOT 2.2, GeneLabs Diagnostics, Singapore).

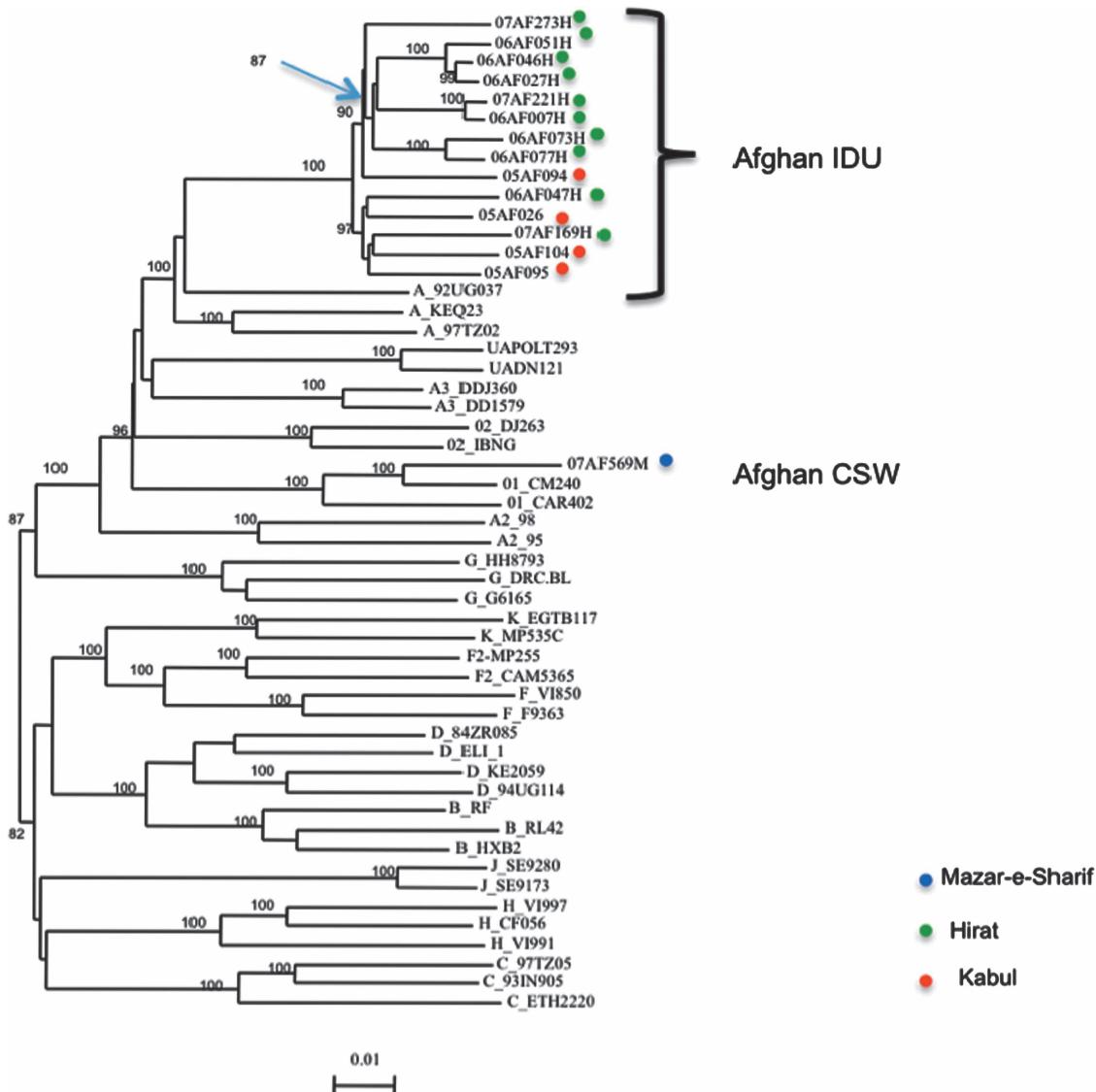


FIG. 1. Full clade tree inclusive of all Afghan genotypes identified to date.

Genotyping of HIV-1 strains was conducted by using primary peripheral blood mononuclear cells (PBMCs) by the same methods described in the earlier Kabul analysis.<sup>10</sup> Descriptive statistics were generated for genotyped samples. Phylogenetic analysis was with PHYLP, including SEQBOOT, DNADIST, NEIGHBOR, and CONSENSE modules, and trees were constructed with TreeTool.<sup>11</sup> Analysis of recombinant forms was performed in stages. Bootscan analysis with reference strains of subtypes A (KEML013), C (97TZ05), D (99UGK09958), and K (MP535C) was performed. The breakpoints across each genome were precisely mapped with recombination breakpoint analysis and visual inspection.<sup>11,12</sup>

Of 623 IDUs recruited in three cities, 11 (1.77%) were HIV positive, all of whom were enrolled in Hirat. In total, 544 SWs were enrolled, of whom only one SW, recruited in Mazar-i-Sharif, was HIV positive (0.18%). The SW in Mazar was female, married, and had lived outside Afghanistan in Pakistan in the past 5 years, but had engaged in sex work only in Afghanistan. One sample from Hirat could not be successfully amplified and analyzed. Of the 10 HIV-positive IDUs from Hirat, all were male, with a mean age of 32.7 years. Most were married (72.7%) and had >6 years of formal education (72.7%). The mean reported duration of injecting was 2.45 years (SD = 1.63 years), with a daily average of two injections. Approximately half (54.5%) reported sharing syringes in the last 6 months.

Complete genetic sequences were obtained from the 11 participants, with Fig. 1 denoting genotypes by clade. The SW participant harbored a CRF01\_AE strain, which bootstrap values indicate varied little from genotypes previously described in Southeast Asia.<sup>13</sup> Of interest, the SW genotype identified did not match that in the IDU networks in Kabul and Hirat; this may indicate limited mixing between IDU and SW networks, although our small sample precludes more definitive inferences.

The 10 samples from the Hirat IDU were all CRF35\_AD. The analyzed subregions (gag p 17 and ENV C1-C5) revealed close homology between the Hirat IDU specimens and with the previously described Kabul specimens (data not shown).<sup>10</sup> The relations among the Hirat strains were also informative. Among these 10, three distinct subclusters comprised two or three strains, whereas two other Hirat strains were generally equidistant from the Kabul strains. One Hirat subcluster had a larger portion of Subtype D genetic material in gp120 when compared with strains in Kabul; the Kabul strains were not part of the three identified subclusters within CRF35\_AD. These data are consistent with more direct and extended chains of HIV transmission in the Hirat IDUs compared with those from Kabul. These data reinforce the connection between incipient epidemics of IDUs in two Afghan cities, Kabul and Hirat, by the identity of circulating HIV subtypes.

With results from an earlier study of Kabul IDUs, the identification of additional CRF35\_AD circulating recombinants among IDUs in Hirat brings the number of complete genomes of this strain from Afghanistan to 14.<sup>10</sup> Previous sequences from Kabul were compared with those described from IDUs in Iran, both in Mashad and in Tehran.<sup>4,5</sup> Although genotypes for IDU in Iran and Afghanistan are quite similar, and most Afghan IDU harboring this strain had been refugees in Iran, where they began injecting drugs, genotype results alone cannot establish strain origin, and conjectures based solely on this evidence are unwarranted.<sup>14</sup> The close phylogenetic linkage between clus-

ters of strains from IDUs in Hirat (Fig. 1) is consistent with a smaller, more interconnected network in that city. The small sample size and lack of information on certain behaviors and, most important, the ascertainment of prevalent infections of unknown duration are limitations of our study.

The molecular epidemiology of HIV transmission in high-risk groups in Afghanistan remains incomplete, providing impetus for further work. Based on the available data, it appears that HIV infection among IDUs is widely but incompletely disseminated in the country, and that the genotypic profile of HIV infection among SWs is difficult to assess because of the low number of observed infections and limited access to this population. The first glimpse of molecular data suggests that IDU and SW networks may not be intimately connected, but these findings warrant further study.

The results indicate that the nascent HIV epidemic among IDUs in Hirat is largely, if not entirely, subtype CRF35\_AD, the same genotype described in Kabul and several sites in Iran.<sup>10,14</sup> The close homology between various samples from Hirat suggests recent infection; thus, harm-reduction programs incorporating voluntary HIV testing and counseling, appropriate provision of antiretroviral therapy, and opioid-substitution therapy and NSPs should be supported in Hirat city, in addition to other affected areas of the country.

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### Author Disclosure Statement

The research study experienced a 6-month lapse of NAMRU-3 IRB approval; this lapse occurred after completion of participant enrollment. The authors have no conflicts of interest.

### Sequence Data

HIV-1 full genome sequences from this study have been submitted to GenBank, and the accession numbers are GQ477441-GQ477451.

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